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MINIREVIEW

Antimicrobial Resistance in Methicillin-Resistant *Staphylococcus aureus* to Newer Antimicrobial Agents

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ABSTRACT Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) result in significant morbidity and mortality for patients in both community and health care settings. This is primarily due to the difficulty in treating MRSA, which is often resistant to multiple classes of antibiotics. Understanding the mechanisms of antimicrobial resistance (AMR) in MRSA provides insight into the optimal use of antimicrobial agents in clinical practice and also underpins critical aspects of antimicrobial stewardship programs. In this review we delineate the mechanisms, prevalence, and clinical importance of resistance to antibiotics licensed in the past 20 years that target MRSA, as well as new drugs in the pipeline which are likely to be licensed soon. Current gaps in scientific knowledge about MRSA resistance mechanisms are discussed, and topics in the epidemiology of AMR in *S. aureus* that require further investigation are highlighted.

KEYWORDS MRSA, antibiotics, antimicrobial resistance

The dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant global health issue that impacts patients in both community and health care settings (1). The Centers for Disease Control and Prevention regards MRSA as a serious threat to public health (2). Understanding the mechanisms of antimicrobial resistance (AMR) in MRSA therefore has great clinical and epidemiological importance. Lowy identified factors that have contributed to the evolution of AMR in MRSA, including the widespread and sometimes inappropriate use of antibiotics, the extensive use of antibiotics as growth enhancers in animal feed, and the relative ease by which MRSA can cross geographic barriers through regional and international travel (3). Livestock, pigs in particular, appear to be important reservoirs for MRSA and fertile breeding grounds for promoting the spread of AMR from animals to humans (4, 5).

Following the discovery and widespread clinical use of methicillin in the early 1960s, *S. aureus* soon became resistant to the drug (6). The primary resistance mechanism was determined to be the expression of the gene *mecA* which encodes PBP2a, a penicillin-binding protein (PBP) with low affinity for β-lactam agents that is spread through horizontal gene transfer (7). Additional auxiliary genes, such as *fem* factors, were also found to be important in the expression of methicillin resistance in *S. aureus* (8, 9). The sequence by which *S. aureus* became resistant to methicillin became a pattern over the following decades as MRSA developed resistance to new agents soon after they were introduced. One exception was vancomycin, as reports of vancomycin-intermediate *S. aureus* (VISA) did not appear until the 1990s (10). The further development of vancomycin-resistant *S. aureus* (VRSA) by a strain already resistant to methicillin occurred through horizontal transfer of the *vanA* gene cluster from vancomycin-resistant *Enterococcus* (11). Fortunately, infections due to VRSA have remained rare in clinical

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TABLE 1 Summary of antibiotic resistance mechanisms in MRSA

Antibiotic class/primary agent	Main mechanism(s) of resistance	Estimated prevalence(s) (%) of resistance in clinical isolates from the U.S. (reference)
β-Lactams (ceftaroline)	PBP2a mutation	1.6 (20)
Lipopeptides (daptomycin)	Mutation in *mprF*	<1
Lipoglycopeptides	Target modification	<1
Oxazolidinones	Point mutations in genes encoding 23S rRNA	<1
Aminoglycosides (plazomicin)	Aminoglycoside-modifying enzymes	<1
Tetracyclines	*tet*(A) and *otr* genes	5 (doxycycline), <1 (newer agents)
Quinolones (delafloxacin)	Mutations in target regions of topoisomerase IV and efflux pumps	7.2 (123)
Pleuromutilins	Target modification and efflux pumps	<1
Mupirocin	*mupA* and *mupB* genes	7 (119)

practice, which may be due the antagonistic effects of *mecA* and *vanA* resistance determinants in *S. aureus* (12).

Because of the myriad mechanisms of AMR that have evolved in MRSA, treatment decisions for MRSA infections can be challenging. This is due in part to the fact that MRSA strains often harbor genes that convey resistance to multiple classes of non-β-lactam agents. In this review we discuss the ways by which MRSA resists antibiotics, with a focus on newer agents, including those in late stages of development. We note gaps in our knowledge about resistance mechanisms in MRSA and recommend directions for further research. A summary of these resistance mechanisms is presented in Table 1. The literature used for this narrative review was identified using PubMed and included reports in English.

RESISTANCE TO β-LACTAM ANTIBIOTICS

Ceftaroline, sometimes called a "fifth-generation" cephalosporin drug, was approved by the U.S. Food and Drug Administration (FDA) in 2010 for the treatment of complicated skin and soft tissue infections (SSTIs) and community-acquired pneumonia. In randomized, controlled trials, ceftaroline was noninferior to comparator drugs (13). MRSA strains carry a large mobile genetic element called SCC*mec*, which in turn carries the *mecA* gene. Conventional β-lactam antibiotics bind to other PBPs, named PBP1, -2, -3, and -4, with various affinities. However, in the presence of PBP2a, the conventional β-lactam drugs are not able to bind effectively to their usual PBP targets. Ceftaroline, as well as a drug called ceftobiprole that has not yet been approved by the FDA, was developed to treat MRSA infections. Ceftaroline is active against MRSA strains because it has a high binding affinity for PBP2a, in contrast to conventional β-lactams (14). As for all β-lactams, binding of PBPs by ceftaroline blocks the ability of these enzymes to catalyze the transpeptidase function that is necessary for staphylococcal cell wall synthesis (15).

Ceftaroline is generally safe and has an adverse effect profile similar to other cephalosporins. It has been used successfully to treat invasive infections in case reports and series both alone (16) and in combination with another active drug, often daptomycin (17). Interestingly, *in vitro* studies demonstrated that the MIC of ceftaroline decreased with increased MICs of vancomycin, daptomycin, and teicoplanin, a phenomenon known as the "seesaw effect" (18). Also, *in vitro*, daptomycin plus ceftaroline showed synergistic killing of *S. aureus* (19). Therefore, combination therapy may be superior to ceftaroline monotherapy, but data are scarce to assess this in human infections.

In studies, >98.4% of MRSA isolates from clinical infections were susceptible to ceftaroline in North America (20) versus 83.3% in Latin America (21), >83% in Europe (22), 16.7% in a small study from 4 countries in Africa (23), and 78.8% in Asia/South Pacific countries (24). The geographic variation in the prevalence of resistance among MRSA may in part be the result of differences in the distribution of strain types of MRSA around the world. For example, ceftaroline resistance was found to be most common in ST239 strains of MRSA both in Australia in 2010 and 2013 (25, 26) and in China in

TABLE 2 Typical PBP2a mutations associated with ceftaroline resistance

Mutation(s)[a]	Reference(s)
Asn104Lys	28
Val117Ile	28
Met122Ile	29
Asp139Trp	24
Asn146Lys	23, 24, 28, 30–33, 113
Glu150Lys	29, 31, 113
Glu170Lys	24
Val117Ile	28
Asn204Lys	23, 24, 30
Asn206Lys	33
Asp208Glu	33
Ser225Arg	23
Ala228Val	28
Thr235Ile	33
Asn236Lys	23
Glu239Lys	24, 30–32, 122
Gly246Glu	23, 31, 113
Lys281Arg	24
His351Asn, H351Gln*	24, 113
Leu357Ile*	28
Tyr446Asn*	115
Glu447Lys*	24, 28, 32, 122
Ile563Thr*	28
Ser649Ala*	28

[a]*, in the penicillin-binding domain.

2011 (27). In a study from Korea in 2017, researchers found that 44% of 159 MRSA isolates obtained from human bloodstream infections were nonsusceptible to ceftaroline (i.e., MIC of $\geq$2), and the majority of isolates were ST72/(SCC*mec* type) IV or ST5/II (28).

Resistance to ceftaroline is usually due to nonsense or nonsynonymous mutations in *mecA*, resulting in changes in the amino acid sequence of PBP2a (i.e., a target protein mutation). Typically in Europe, isolates with an MIC of $>$8 mg/liter with single mutations have mutations in the transpeptidase pocket on PBP2a and belong to ST228, ST239, ST22, and ST5, while those with a lower MIC of 2 have non-PBD mutations (29). Of 458 isolates collected during 1985 to 1987 or 2006 to 2013 from animals or humans in the United Kingdom, only 3 were identified, all from the 2006–2008 period, with mutations previously associated with ceftaroline resistance; they were ST241/II (CC8) or ST22/IVh (CC22) (30). In a Swiss study, among 60 isolates archived in 1998 to 2004, 40 had an MIC of 2 mg/liter or greater, and most of these were ST228 or ST247. All had missense mutations in the allosteric binding domain of *mecA* (Asn146Lys, Glu239Lys, or a combination of three mutations: Asn146Lys plus Glu150Lys plus Gly246Glu) (31). Among 8,037 *S. aureus* isolates collected globally in 2010, 4 were found to have a ceftaroline MIC of $>$2 mg/liter. Three were from Thailand, and one was from Spain. Isolates with an MIC of 2 mg/liter had a Glu239Lys mutation in PBP2a, while those with MIC of 8 mg/liter had this mutation along with a second mutation, Glu447Lys. Glu447Lys falls within the PBD of PBP2a, while Glu239Lys does not (32) (Table 2). In a study from 15 large hospitals in Russia from 2010 to 2014, 21 isolates (all ST228 or ST239) with an MIC of 2 mg/liter had one or more *mecA* mutations (Table 2). In three additional ST8 MRSA isolates with a ceftaroline MIC of 2 mg/liter, *mecA* was wild type (33), indicating a mechanism of resistance independent of PBP2a mutation. Among 11 isolates from Nigeria (all ST15 or ST241) in 2007 to 2012, ceftaroline resistance was associated with one of three missense mutations in *mecA* (Asn146Lys, Asn204Lys, or Gly246Glu) (23). In a 2017 study from South Korea, ceftaroline-resistant strains carried one or more of eight different mutations in *mecA*, four of which were in the PBD and four were not. These authors found a greater MIC associated with a greater number of mutations (28).

Interestingly, the Glu447Lys mutation in *mecA* developed with *in vitro* passaging of

the SF8300 USA300 MRSA strain in the presence of ceftaroline, yielding an isolate with low-level resistance. When COL, a commonly used laboratory strain, was passaged in ceftaroline, high-level ceftaroline resistance (32 or 64 μg/ml) developed with mutations in *pbp2* (coding for PBP2), *pbp4* (coding for PBP4), and *gdpP*, but surprisingly not in *mecA* (34), again suggesting a mechanism of resistance unrelated to PBP2a mutations. Similarly, another ceftaroline passaging study resulted in some strains developing resistance with no changes in the *mecA* gene, while others did develop a wide variety of single and double *mecA* mutations (35).

Therefore, most ceftaroline resistance is due to mutations in *mecA*. There is likely a risk of resistance emerging during therapy with ceftaroline, and there are mechanisms of resistance that are not yet understood. In addition, there is some evidence that mutations in the promotor of PBP4, yielding increased production of PBP4, may result in resistance to ceftaroline (36, 37).

RESISTANCE TO LIPOPEPTIDES

Daptomycin, currently the only available lipopeptide, was first approved in the United States in 2003 and has *in vitro* bactericidal activity against many Gram-positive bacteria. It quickly became the main alternative to vancomycin for serious MRSA infections, such as bacteremia and endocarditis (38). However, reports about the emergence of daptomycin-nonsusceptible MRSA strains during the course of treatment are concerning and have important therapeutic implications (39, 40). The basis for reduced susceptibility to daptomycin in MRSA has not been fully elucidated. Since the MIC that determines resistance in daptomycin has not yet been established, the term "nonsusceptible" is preferred by some investigators over "resistant" (41). Even before the drug was approved, Silverman et al. observed the emergence of daptomycin-nonsusceptible mutants following passage through increasing concentrations of daptomycin (42). Subsequent work identified a number of changes in the cytoplasmic membranes of nonsusceptible strains, including enhanced membrane fluidity, increased net positive surface charge, reduced susceptibility to daptomycin-induced depolarization, and lower surface binding of daptomycin (43).

The gene *mprF* encodes an enzyme called lysyl-phosphatidyl glycerol synthetase, which transfers positively charged lysine molecules and adds them to phosphatidyl glycerol in the cell membrane (44). When *mprF* is mutated, lysyl-phosphatidyl glycerol increases in the outer layer of the cell membrane, leading to an increased positive charge which reduces susceptibility to daptomycin (45). However, only certain mutations in *mprF*, such as the T345A single nucleotide polymorphism, reproducibly decrease daptomycin susceptibility, likely as a result of reduced intramolecular interactions (45). Mutations in the *mprF* gene are the most common mutation seen in MRSA strains with reduced daptomycin susceptibility. However, *mprF* mutations result in a high fitness cost, such that daptomycin-nonsusceptible strains can revert to daptomycin susceptible when antibiotic pressure is removed (46). There have been a number of studies examining pairs of isolates that became less susceptible to daptomycin with therapy, and several candidate genes have been identified as potentially associated with the reduced susceptibility phenotype (Table 3). For example, inactivation of the genes *dsp1* or *asp23* leads to reduced daptomycin susceptibility, whereas overexpression of one or both causes increased susceptibility (47). Furthermore, the expression of the *dltA* gene is significantly downregulated by daptomycin (48). Although these mechanisms remain only partly understood, further investigation may lead to the development of inhibitors that block the development of resistance to daptomycin in MRSA.

Therapy with daptomycin may also lead to changes in thickness in the cell wall of MRSA (49). Using transmission electron microscopy, Kanesaka et al. found some strains that were exposed to daptomycin and became resistant developed increased thickness of their cell wall and when they reverted back to daptomycin susceptible, their cell wall thickness decreased to the same level as daptomycin-susceptible MRSA (50).

TABLE 3 Major putative genes associated with resistance to daptomycin in MRSA[a]

Gene	Role in MRSA metabolism/virulence	Reference	Comments
mprF	Associated with a gain of enzymatic function, resulting in an increase in the positive charge of the cell membrane	45	*mprF* mutations are the most frequently reported genetic lesions in DAP-NS MRSA isolates
asp23	Stress response gene that encodes an alkaline-shock protein; loss of function increases tolerance to DAP and VAN	47	
dsp1	Encodes hypothetical lipopeptide; overexpression increases net positive charges in cell membrane	47	
dltA	Incorporation of D-alanine into cell wall teichoic acids; contributes to the staphylococcal net positive surface charge	48	
vraSR	Regulates the cell wall biosynthesis pathway	49	A causal relationship between point mutation of *mprF* and increased expression of *vraSR* may explain why daptomycin resistance is often present with vancomycin resistance in clinical isolates

[a]VAN, vancomycin; DAP, daptomycin; DAP-NS, daptomycin nonsusceptible.

RESISTANCE TO LIPOGLYCOPEPTIDES

The three lipoglycopeptides available in the United States—dalbavancin, oritavancin, and telavancin—are semisynthetic derivatives of glycopeptides (vancomycin and teicoplanin). The glycopeptides' common heptapeptide core binds to D-alanyl-D-alanine (D-Ala-D-Ala) termini of growing peptidoglycan chains, ultimately inhibiting bacterial cell wall synthesis (51). Lipoglycopeptides are more potent than vancomycin due to unique structural modifications to each drug's heptapeptide core. All three lipoglycopeptides contain lipid side chains that anchor the drug to the cell membrane, thereby providing stability and increasing local drug concentrations. For oritavancin and telavancin, interactions with the cell membrane facilitate a second mechanism of action via concentration-dependent cell membrane depolarization leading to increased permeability. The structure of oritavancin allows for additional mechanisms of action, including binding to a secondary site in peptidoglycan chains, the pentaglycyl bridging segment of lipid II, inhibiting transpeptidation; it may also inhibit RNA synthesis (51, 52).

Target modification is the most common mechanism of resistance against glycopeptides and lipoglycopeptides. Seven known resistance elements (VanA, -B, -C, -D, -E, -G, and -L) modify the structure of the peptidoglycan precursors; of these, VanA resistance is the only one found in *S. aureus* isolates and is the main resistance mechanism for VRSA. The plasmid-borne transposon Tn*1546* confers VanA resistance by encoding 9 proteins that ultimately modify the D-Ala-D-Ala termini of peptidoglycan chains to D-Ala-D-lactate, inhibiting target binding by vancomycin, telavancin, and dalbavancin. Oritavancin retains *in vitro* activity against VRSA, likely because of its multiple mechanisms of action (53). VanA gene expression is inducible; all three lipoglycopeptides activate the VanA operon.

Vancomycin-intermediate *S. aureus* (VISA; vancomycin MIC of 4 to 8 μg/ml) and heterogenous VISA (hVISA; stains with subpopulations that exhibit vancomycin MICs of $>$2 μg/ml) are more common than VRSA and emerge with prolonged vancomycin exposure. Evidence suggests that the accumulation of point mutations in several regulatory systems allow for the emergence of VISA and hVISA by remodeling and thickening the bacterial cell envelope (54). All three lipoglycopeptides remain active *in vitro* against VISA and hVISA strains (53).

Lipoglycopeptide resistance among *S. aureus* remains rare (55). A surveillance study conducted in the United States and Europe from 2010 to 2014 showed that 99.9% of *S. aureus* isolates were susceptible to oritavancin (56). A global surveillance study conducted in 2002 to 2012 showed that 99.8% of multidrug-resistant MRSA isolates were susceptible to dalbavancin (57).

Resistance in clinical isolates has been reported, most recently for dalbavancin. The structure of dalbavancin contains a long lipophilic side chain that extends its half-life, allowing for once-weekly dosing; however, this may also allow for the emergence of resistance when organisms are exposed to subtherapeutic levels of the drug. In one

report, dalbavancin-nonsusceptible *S. aureus* small-colony variants emerged during a 30-week course of therapy for device-related infective endocarditis (58). Structural analysis revealed increased cell wall thickness and abnormal cell wall construction in dalbavancin nonsusceptible isolates compared to the wild type. In another report, dalbavancin exposure induced both vancomycin and dalbavancin nonsusceptibility; whole-genome sequencing identified a single mutation in the *yvqF* gene (59). As lipoglycopeptide off-label use continues to increase, regular surveillance for the emergence of resistance to these drugs is critical.

RESISTANCE TO OXAZOLIDINONES

The oxazolidinones are synthetic antibiotics that prevent bacterial protein synthesis by blocking the formation of a functional 70S initiation complex. Both licensed drugs in this class, linezolid and tedizolid, bind to the bacterial 23S rRNA (rRNA) at the ribosomal peptide-transferase center, interrupting transitional RNA positioning. Although structurally similar to linezolid, tedizolid's design allows for enhanced interactions at the binding site, accounting for its increased potency and retained activity despite, in some cases, linezolid resistance (60).

All three well-described mechanisms of resistance to this class of drugs alter the oxazolidinone binding site. Point mutations in the genes encoding the 23S rRNA are most common, with most mutations occurring in the central loop of domain V of the 23S rRNA (54). Most bacteria have multiple copies of the 23S rRNA gene (*S. aureus* has four to seven), and the accumulation of mutations determines the degree of linezolid resistance (i.e., the mutant-gene dosage effect) (61, 62). The first clinical isolate of linezolid-resistant MRSA had the same mutation, G2576T, in all five copies of its 23S rRNA gene (61), and mutations in this gene are most the commonly reported in prevalence studies. Although less common, mutations in the genes encoding L3 and L4 ribosomal proteins also confer linezolid resistance, likely by inducing a similar spatial change in the linezolid binding site as 23S rRNA mutations. A study using cryo-electron microscopy showed that one amino acid deletion in L3 induced a structural rearrangement of the linezolid binding site that included repositioning several of the 23S rRNA bases frequently targeted by point mutations (63).

Acquisition of the *cfr* (chloramphenicol-florfenicol resistance) gene by *S. aureus* confers resistance to several antibiotics, including linezolid, by encoding a rRNA methyltransferase that alters position A2503, obstructing the drug binding site at the ribosomal peptide-transferase center. Point mutations in the genes encoding the 23S RNA, L3, and L4 ribosomal proteins emerge with linezolid exposure, but resistance conferred by the *cfr* gene can occur without prior exposure to the drug. Cfr has been associated with various mobile genetic elements, as well as outbreaks of linezolid-resistant *S. aureus* worldwide (64). Several bacterial species in the human and livestock commensal flora harbor the *cfr* gene, serving as a reservoir for drug resistance with the potential to spread. In one study of MRSA isolates from humans and animals in China, 20 of 128 isolates were *cfr* positive; *cfr*-positive isolates were more likely to harbor additional antibiotic resistance genes compared to *cfr*-negative isolates (65).

Whole-genome sequencing continues to uncover new resistance genes. The *optrA* gene, which confers resistance to oxazolidinones and phenicols, was first described in enterococcus species in 2015 (66). This transferrable gene has since been detected in MRSA isolates where it commonly coexists with the *cfr* gene (66). Florfenicol use in animals in China may have selected for the emergence of both *cfr* and *optrA* (67). A surveillance study identified genetically related *optrA*-positive isolates of enterococci from the same institution, highlighting this gene's potential for spread among Gram-positive organisms (68). Its mechanism of action remains unknown. Genetic studies defined the *optrA* gene as an ATP-binding cassette transporter, a gene superclass whose products mediate the influx and efflux of drugs, among other molecules, across bacterial cells and organelles. However, unlike other members of this class, *optrA* lacks transmembrane domains; it likely acts via ribosomal protection (67, 69, 124). Another novel gene, *poxtA*, was first identified in a MRSA isolate, shares some structural

similarities with the *optrA*, and confers *in vitro* resistance to oxazolidinones, phenicols and tetracyclines (70). The prevalence of the *poxtA* gene among MRSA isolates and its mechanism of resistance are unknown.

Oxazolidinone resistance among *S. aureus* remains rare; a 2011 to 2015 U.S. surveillance study found only 14 of 15,177 *S. aureus* isolates exhibited linezolid-nonsusceptible MIC values (71). A similar study detected tedizolid *in vitro* nonsusceptibility (defined as an MIC of >0.5 mg/liter) in only 19 of 7,813 *S. aureus* isolates from the United States and Europe between 2009 and 2013 (72). Organisms may acquire more than one resistance mechanism; the LEADER study detected more than one resistance mechanism in 4 of the 14 linezolid-resistant *S aureus* isolates, all 4 of which had an linezolid MIC of ≥8. Tedizolid resistance data are limited because this drug was only first licensed in the United States in 2015. Given its increased potency, tedizolid remains active despite linezolid resistance in many cases and is unaffected by the presence of the *cfr* gene (72). However, it may not be active against *optrA*-positive isolates.

RESISTANCE TO AMINOGLYCOSIDES

Aminoglycosides disrupt bacterial protein synthesis by binding to the A-site on the 16S rRNA of the 30S ribosome, altering its conformation and promoting the mistranslation of the tRNA. Some aminoglycosides also block bacterial protein synthesis by inhibiting the initiation and/or elongation phase of this process (73). Resistance and toxicity limit the role of this class in the management of MRSA infections.

Aminoglycoside modifying enzymes are the most common mechanism of resistance to aminoglycosides, especially in *S. aureus*. These enzymes inactivate aminoglycosides by acetylating, phosphorylating, or adenylating amino or hydroxyl groups of the antibiotic structure. More than 100 aminoglycoside modifying enzymes have been described and are encoded by genes commonly found on plasmids and transposons, including those harboring resistance to other antibiotic classes (74). Three of these enzymes, ANT(4′)Ia nucleotidyltransferase, bidomain AAC(6′)Ie-APH(2′)Ia acetyltransferase and phosphotransferase, and APH(3′)IIIa phosphotransferase, confer resistance to one or more aminoglycosides used in clinical practice, including gentamicin, tobramycin, and amikacin and are common among MRSA isolates, although their relative prevalence varies geographically (75). Due to structural differences, plazomicin, a next-generation synthetic aminoglycoside, retained *in vitro* activity against 55 MRSA isolates that expressed one or more aminoglycoside-modifying enzymes (76). Plazomicin is not protected against other mechanisms of resistance, including 16s rRNA methyltransferases that directly modify the aminoglycoside target site, but these enzymes have not been reported in *S. aureus* (77).

Genomewide studies continue to reveal additional mechanisms of resistance to aminoglycosides. One such study described mutations in several different genes encoding elements of the electron transport chain that lead to a reduction in membrane potential, a change that is associated with reduced aminoglycoside uptake and a modest increase in MIC and is often associated with a small-colony variant phenotype (78).

RESISTANCE TO TETRACYCLINES

There are a number of mechanisms by which MRSA acquires resistance to the tetracycline class of antibacterial drugs, which were comprehensively reviewed by Nguyen et al. (79). Tetracycline resistance primarily results from the acquisition of *tet* (tetracycline resistance) and *otr* (oxytetracycline resistance) genes, more than 30 of which have been identified with a variety of resistance mechanisms. The most common of these, *tet*(A), conveys resistance to both doxycycline and tetracycline. Doxycycline is commonly used for treating SSTIs due to MRSA, especially in outpatient settings or for oral step-down therapy. In community-associated (CA) MRSA strains that are resistant to doxycycline, including MDR strains of the USA300 clone, the predominant resistance gene is *tet*(K), which codes for an efflux pump (80). Minocycline susceptibility is not affected by *tet*(K), even after incubation in subinhibitory concentrations of the drug (79). The *tet*(K) gene, which is plasmid-borne, has been shown to spread through a

USA400 MRSA strain (81). The *tet*(M) gene is a chromosomal gene that enhances ribosomal protection by encoding elongation factor-like proteins. Investigators found that in surveillance and clinical cultures of MRSA isolates from military personnel who received doxycycline for malaria prophylaxis, there was no significant difference in tetracycline resistance between isolates collected from patients with or without antimalarial prophylaxis (82). Notably, more of the isolates in the doxycycline exposure group had *tet*(M) resistance genes ($P = 0.031$), suggesting that *tet*(M) resistance in these MRSA strains might be subclinical.

Tigecycline was designed to overcome resistance to other drugs in the tetracycline class through enhanced affinity for its binding sites compared to other tetracyclines. The presence of a bulky side chain at carbon 9 provides steric hindrance, preventing the Tet efflux protein from exporting tigecycline out of the bacterial cell (83). Dabul et al. found that tigecycline resistance was induced in an MRSA clinical isolate by increased efflux of the drug due to mutations in the transcriptional regulator MepR and in the efflux pump MepA (84). Furthermore, tigecycline resistance has been observed in a MRSA isolate with mutations in the *rpsJ* gene, which encodes the ribosomal S10 protein (85). This MRSA isolate came from a patient with cystic fibrosis who had received successive courses of antibiotics including minocycline, but not tigecycline. These reports are of concern, especially in light of a global surveillance study that included 5,118 *S. aureus* isolates collected from 2010 to 2014 which found the MIC_{90} to tigecycline was 0.25 mg/liter, and no resistant isolates were detected (86). Surveillance for tigecycline-resistant MRSA strains should remain an ongoing priority.

Eravacycline is a fluorinated glycylcycline similar in structure to tigecycline. Zhang et al. reported excellent *in vitro* activity ($MIC_{50} \leq 0.25$ mg/liter) for eravacycline against MRSA, including isolates harboring *tet* resistance genes (87). Eravacycline was shown to retain activity against strains overexpressing MepA (MICs of $\leq$0.016 μg/ml), compared to an increase in the tigecycline MIC from 0.016 to 1 mg/liter (88). Using a mouse septicemia model, Grossman et al. found the protective antimicrobial doses for 50% of the infected animals (PD_{50}s) against MRSA with *tetM* and MRSA with *tetK* to be 1.0 mg/kg (95% confidence interval [CI], 0.56 to 1.4) and 0.3 mg/kg (95% CI, 0.13 to 0.47), respectively (89). In both MRSA isolates, eravacycline and tigecycline had similar PD_{50} values with overlapping 95% CIs.

Omadacycline is an aminomethylcycline derived from the tetracycline class that was recently approved for the treatment of community-acquired pneumonia and acute bacterial skin and skin structure infections in adults (90, 91). It has an aminomethyl substituent at the C-9 position of the core six-member ring, conveying the ability to overcome ribosomal protection proteins and efflux pump mechanisms. For example, omadacycline is unaffected by the presence of the *tet*(K) efflux gene, the ribosome protection *tet*(M) gene, or the ribosome protection protein Tet(O) (92). Compared to doxycycline, MRSA isolates with known tetracycline resistance determinants showed lower MICs by broth microdilution for omadacycline (0.12 to 2mg/liter) independent of the mechanism of resistance [*tet*(K), *tet*(M), or *tet*(K) plus *tet*(M)] (93). In a surveillance study from North America and Europe that included approximately 200 clinical isolates of MRSA, isolates with MICs of 2 and 4 μg/ml were observed in 2010, but none were detected in 2014, supporting a lack of emerging resistance to omadacycline (71). However, these results should be interpreted with caution. Omadacycline was approved by the FDA in 2018, and its escalating use will invariably lead to an increase in resistance through evolution, whereby resistant mutants arise stochastically in bacterial populations and expand under the selective pressure from antibiotic therapy (94).

RESISTANCE TO QUINOLONES, WITH A FOCUS ON THE NOVEL AGENT DELAFLOXACIN

Fluoroquinolones (FQs), a class of fully synthetic antibiotics, were first introduced into clinical practice in 1962 with the development of nalidixic acid. Their use increased rapidly in the late 1990s after the introduction of ciprofloxacin. They are active against

a broad range of Gram-negative and Gram-positive species and have a role in the therapy of multidrug-resistant tuberculosis and nontuberculous mycobacterial infections.

In 2017, delafloxacin, a nonzwitterionic FQ, was approved by the FDA to treat acute bacterial skin and skin structure infections (ABSSSIs) with both enteral and intravenous preparations available (95). Delafloxacin has lower MICs against *S. aureus* than other FQs and also has a higher barrier to resistance. Thus, unlike other FQs, it may serve as an effective antistaphylococcal drug used as monotherapy. In studies of *S. aureus* obtained from clinical studies in 2014, delafloxacin was effective against up to 99.5% of MSSA strains from the United States and Europe combined, 95.3% of MRSA strains from Europe, and 91.2% of MRSA strains from the United States (96). In addition to *S. aureus*, the drug is effective against *Streptococcus pneumoniae*, anaerobic bacteria, *Neisseria gonorrhoeae*, *Ureaplasma* sp., *Legionella*, *Chlamydia pneumoniae*, and *Mycoplasma* sp., among many other species. Activity against the enterococci is variable (95).

Delafloxacin can form complexes between DNA and either topoisomerase IV or DNA gyrase, and it is thus described as a "dual-targeting" FQ. Inhibition of either or both of these enzymes may result in bacterial cell death by producing DNA double-strand breaks, which enzymes of the bacterial cell cannot repair. Delafloxacin is more potent against Gram-positive organisms than other FQs, perhaps in part because it remains anionic at neutral pH due to a substitution of the R7 position (3-hydroxy-1-azetidinyl) (95). As an anionic molecule, compared to other FQ drugs, delafloxacin can more readily diffuse into and accumulate within bacteria, where it is retained due to transition to its ionic form at the neutral intracellular pH (97). This characteristic also makes the antibiotic more effective in acidic environments (98). It has activity against biofilm-related infections (99, 100) and intracellular bacteria (97, 98), but this activity likely depends upon the ambient pH (100).

Delafloxacin exposure at low concentrations *in vitro* rarely selects resistant mutants of *S. aureus* (estimated rate, 2×10^{-9} to 9.5×10^{-11}) (101). Also, the estimated concentration of the delafloxacin that selects for resistant mutants (i.e., the mutant selection window) is 8 to 32 times lower than for other FQs (98), a difference that may result from the drug's dual-targeting mechanism of action.

Resistance to the FQs, including delafloxacin, often involves point mutations in the target enzymes or the action of efflux pumps in bacterial cells. In *S. aureus*, resistance is usually mediated by point mutations in the ParC subunit of topoisomerase IV. Delafloxacin often retains potency against *S. aureus* resistant to other FQ drugs due to target gene mutations or modifications. This relative resistance seems related to the structure of delafloxacin (perhaps due to C-7 and C-8 substitutions); delafloxacin resistance occurs only with several mutations in the target regions of topoisomerase IV (95, 101). It is believed that delafloxacin remains active in the setting of one or only a small number of mutations because its intrinsic activity is so much greater than that of other FQs (98).

Common *S. aureus* efflux pumps active against FQs, which may result in a resistant phenotype, include NorA, NorB, NorC, MdeA, QacA, and QacB (102–104). The antiseptic chlorhexidine gluconate is also removed from cells by the plasmid-encoded efflux pumps QacA and QacB, sometimes called antiseptic resistance genes, and acquisition of these pumps in a *S. aureus* population may be coselected by use of chlorhexidine or FQs (102). Delafloxacin is not as active a substrate for typical *S. aureus* efflux pumps compared to other drugs in the class (101), and thus resistance resulting from the presence of these pumps is less likely for delafloxacin than for other FQs.

RESISTANCE TO PLEUROMUTILINS, A NOVEL CLASS OF ANTIMICROBIALS

Pleuromutilins are a class of antibacterials first isolated in 1951 from a fungus called *Pleurotus mutilis* (now renamed *Clitopilus scyphoides*) (105). The molecule of the natural product pleuromutilin, an effective antibacterial against Gram-positive bacteria, was subsequently modified. By 2019, hundreds of related compounds had been engineered in the search for safe and effective antibiotics. In 1979, tiamulin was approved for veterinary use and it has since then been used in livestock to treat respiratory and

gastrointestinal disease. In 1999 valnemulin, a second veterinary systemic pleuromutilin antimicrobial, was approved and has since also been widely used in Europe and Asia (106). Retapamulin was approved in 2007 only for topical use in decolonization of MRSA and for the treatment of impetigo, a superficial, honey-crusted staphylococcal skin infection (107).

A novel pleuromutilin drug effective against most MRSA strains, lefamulin, was synthesized in 2006 (105) and is being developed for systemic human use. In a phase 2 randomized, controlled trial for ABSSSIs lefamulin was noninferior to intravenous vancomycin (108). In August 2019 the FDA approved it for treatment of adults with community-acquired bacterial pneumonia.

Pleuromutilins act by inhibiting the 50S subunit of the ribosome, binding at a site called the peptidyl transferase center (107), thereby interfering with protein synthesis. They specifically inhibit initiation of translation (105). Mechanisms of resistance to pleuromutilins have been studied in livestock-associated MRSA strains given the extensive use for decades of tiamulin and valnemulin (106, 109). Despite widespread use in agriculture, resistance to lefamulin in 2015 and 2016 remained rare, identified in only 0.3% of 2,919 *S. aureus* isolates from a global collection obtained from human cultures (110).

Resistance to pleuromutilins in *S. aureus* may be caused by target modification, ribosomal protection, or efflux; inactivation by modification of the drug has not been described (109). One mechanism involves alteration of the target site on the ribosome. Although it may require three or more mutations to result in a resistant phenotype (111), often these include a nonsynonymous mutation resulting in the Asn446Asp amino acid substitution in ribosomal protein L3 (coded for by *rplC*) (105).

Resistant clones may emerge when *S. aureus* acquires certain new genes by horizontal transfer. These include the plasmid-transferable *cfr* gene, which methylates a specific site on 23S rRNA (carbon 8 of base A2503). This methylation by the *cfr* gene product results in resistance to several classes of antibiotics, including lincosamides, streptogramin, phenicols, pleuromutilins, and linezolid ($PhLOPSa_A$) (112). Because of the frequent use of pleuromutilins in livestock, resistance due to *cfr* has been identified in *S. aureus* in many studies in China and Europe (106). A third cause of pleuromutilin resistance in *S. aureus* is the family of at least four *vga* genes with variants, including *vga*(*A*)$_V$, *vga*(*A*), *vga*(*C*), and *vga*(*E*), as well as *lsa*(*E*) that likely all result in ribosomal protection (106, 109, 110, 124). *vga*(*A*) may be transmissible among strains since it is can be carried on a transposon or a plasmid (109). It has been identified in ST398 livestock-associated MRSA strains (113), as has *vga*(*C*), which may also be carried on a plasmid (114). The spread of mobile genetic elements among animal and human *S. aureus* strains raises concern for the emergence of widespread pleuromutilin resistance among human strains if drugs in this class are widely used.

RESISTANCE TO MUPIROCIN

Although mupirocin was first discovered in the 1970s, its use became widespread as a decolonizing agent with the emergence of the CA-MRSA epidemic in the United States during the 1990s. Reports of increasing resistance to mupirocin by MRSA soon followed (115, 116). The *ileS-2* gene was determined to be responsible for conveying mupirocin resistance (117). Subsequently, high-level resistance to mupirocin was found to be conferred by the *mupA* and *mupB* genes, which encode novel isoleucyl-tRNA-synthetases and are carried by plasmids (118). The REDUCE-MRSA study was a three-arm, cluster-randomized trial that evaluated screening, isolation, and decolonization with chlorhexidine and mupirocin in intensive care unit patients (119). Of the 3,173 isolates analyzed, at baseline 7.1% of MRSA isolates expressed low-level mupirocin resistance and 7.5% expressed high-level mupirocin resistance (119). Mupirocin remains the best option for MRSA nasal decolonization, although reports of increasing resistance and treatment failure are worrisome. Therefore, the pursuit of novel decolonization agents should be an ongoing priority. Another option is to develop agents that act synergistically with mupirocin, one of which was recently described (120).

CONCLUSIONS

The ability of MRSA to develop resistance to every antibiotic to which it is exposed makes it a formidable threat to human health. Resistance mechanisms have been present in bacteria for millennia, while antibiotics have been in clinical use for approximately 80 years. Faced with selective pressure from increasing antibiotic use, bacteria have adapted and developed complex mechanisms in order to survive. This, along with decreasing interest in antibiotic development by the pharmaceutical industry, makes it clear that preserving our current antibiotic armamentarium through wise antibiotic stewardship is paramount. Further development of novel compounds such as teixobactin (121), identification of additional drug targets, better stewardship, and more informed choices about combination therapy will hopefully allow us to continue to treat MRSA infections for the foreseeable future.

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